Electronic Supporting Information

Development of a Conformational Histamine H_3 receptor Biosensor for the Synchronous Screening of Agonists and Inverse Agonists.

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Supplementary Table 1. Summary of H_3R ligand affinities/potencies and efficacies assessed with different assays

Compound	Affinity for wildtype H_3R (binding assay; $pK_{D/i}$ ± s.d.)	Affinity for Δicl3 $H_3R_{Nluc/Halo(618)}$ (binding assay; $pK_{D/i}$ ± s.d.)	Potency at Δicl3-$H_3R_{Nluc/Halo(618)}$ (conformational assay; pEC_{50} ± s.d.)	Relative E_{max} at wildtype H_3R (type of assay)	Relative E_{max} at Δicl3-$H_3R_{Nluc/Halo(618)}$ (normalized to histamine ± s.e.m.)
[^{3}H] NAMH	8.9 ± 0.1	8.7 ± 0.2	n.d.	1.0 of histamine [4] (CRE reporter gene)	n.d.
histamine	6.3 ± 0.2 [1]	7.4 ± 0.1	6.7 ± 0.0	1.0 of histamine [4] (CRE reporter gene)	1.00 ± 0.02 *
RAMH	8.4 ± 0.1 [2]	8.3 ± 0.0	n.d.	1.0 of histamine [4] (CRE reporter gene)	n.d.
SAMH	7.6 ± 0.1 [2]	7.3 ± 0.1	n.d.	1.0 of histamine [4] (CRE reporter gene)	n.d.
imetit	8.3 ± 0.4 [1]	9.1 ± 0.1	8.2 ± 0.1	1.0 of histamine [4] (CRE reporter gene)	0.61 ± 0.01 *
impentamine	8.3 ± 0.1 [3]	8.6 ± 0.0	7.9 ± 0.2	0.9 of NAMH [3]	0.19 ± 0.02
VUF5207	7.8 ± 0.2 [3]	7.4 ± 0.0	7.8 ± 0.7	0.7 of NAMH [3]	0.10 ± 0.01
VUF4904	7.9 ± 0.1 [3]	7.7 ± 0.1	6.3 ± 0.2	-0.1 of NAMH [3]	-0.25 ± 0.04
VUF4903	8.0 ± 0.0 [3]	8.1 ± 0.1	6.5 ± 0.1	-0.6 of NAMH [3]	-0.46 ± 0.02

Compound	Affinity for wildtype H_3R (binding assay; $pK_{D/i}$ ± s.d.)	Affinity for Δicl3 $H_3R_{Nluc/Halo(618)}$ (binding assay; $pK_{D/i}$ ± s.d.)	Potency at Δicl3-$H_3R_{Nluc/Halo(618)}$ (conformational assay; pEC_{50} ± s.d.)	Relative E_{max} at wildtype H_3R (type of assay)	Relative E_{max} at Δicl3-$H_3R_{Nluc/Halo(618)}$ (normalized to histamine ± s.e.m.)
UR-PI294	9.0 ± 0.1 [5]	8.7 ± 0.1	8.5 ± 0.5	0.4 of histamine [5] (GTPase assay)	0.07 ± 0.01
clobenpropit	9.6 ± 0.1 [1]	9.3 ± 0.1	7.4 ± 0.0	-0.8 of histamine [4] (CRE reporter gene)	-0.38 ± 0.01
thioperamide	7.3 ± 0.3 [1]	7.2 ± 0.1	7.1 ± 0.1	-0.8 of histamine [4] (CRE reporter gene)	-0.30 ± 0.01
pitolisant	8.6 ± 0.0 [1]	8.0 ± 0.1	7.2 ± 0.0	-0.4 of histamine (CRE reporter gene)	-0.48 ± 0.01 [#]
Z27743747	7.4 [6]	7.3 ± 0.1	6.5 ± 0.1	unknown	-0.65 ± 0.02 [#]
Z3303614736	6.8 [6]	6.7 ± 0.1	6.5 ± 0.1	unknow	-0.53 ± 0.02

Non-referenced values were assessed in the present study. n.d.: not determined.
Values are mean ± s.d. of at least three independent experiments.
*/[#]: Statistically significant difference between E_{max} of histamine and imetit (*) and between pitolisant and Z27743747 ([#]) in the conformational BRET assay. Significance was assessed applying extra-sum-of-squares F test ($p < 0.0001$).

References:

(1) Mocking, T.A.M., Verweij, E.W.E., Vischer, H.F., and Leurs, R. (2018). Homogeneous, Real-Time NanoBRET Binding Assays for the Histamine H3 and H4 Receptors on Living Cells. Mol Pharmacol 94, 1371-1381.

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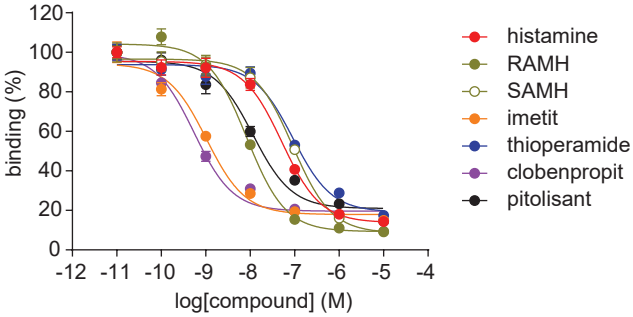
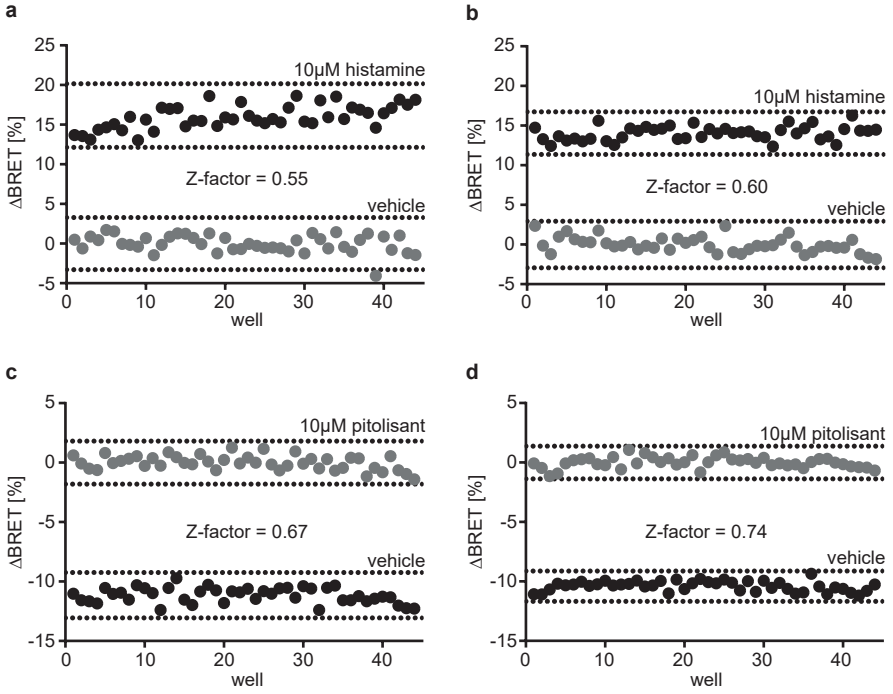
(6) Schaller, D., Hagenow, S., Stark, H., and Wolber, G. (2019). Ligand-guided homology modeling drives identification of novel histamine H3 receptor ligands. PLoS One 14, e0218820, https://doi.org/10.1371/journal.pone.0218820.

Supplementary Figure 1.



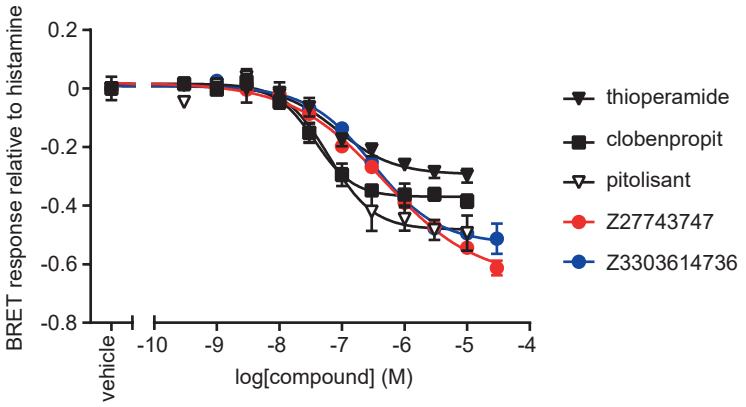
Competition binding of selected unlabelled H_3R ligands with [^{3}H]NAMH to cells stably expressing Δicl3-$H_3R_{NlucHalo(618)}$.

Supplementary Figure 2.



BRET signals and Z-factors of Δicl3-$H_3R_{Nluc/Halo(618)}$ assessed in four independent 96-well plates using histamine (**a**, **b**) or pitolisant (**c**, **d**) as positive controls to determine the screening windows for H_3R agonists and inverse agonists, respectively.

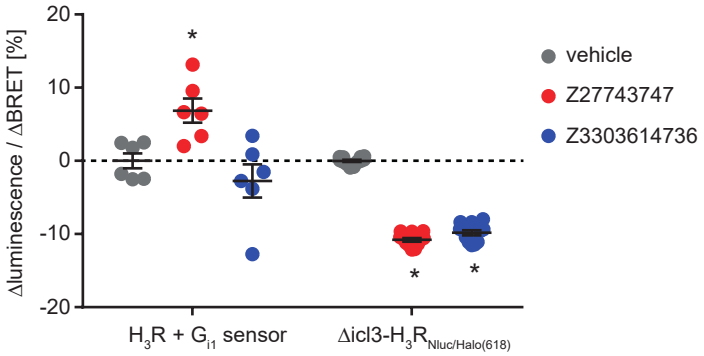
Supplementary Figure 3.



Concentration response curves of H_3R inverse agonists assessed with Δicl3-$H_3R_{Nluc/Halo(618)}$ and normalized to the maximum histamine response.

Supplementary Figure 4.

a



b

	S/N ratio	
	G_{i1} sensor	$\Delta icl3\text{-}H_3R_{Nluc/Halo(618)}$
Z27743747	3	21
Z3303614736	-1	20

Comparison of assay sensitivity without H_3R agonist pre-stimulation. **a**) Luminescence and BRET responses induced by Z27743747 and Z3303614736 in cells expressing H_3R wildtype along with the split Nluc-based G_{i1} sensor or the conformational H_3R sensor $\Delta icl3\text{-}H_3R_{Nluc/Halo(618)}$, respectively. Statistical differences were assessed by one-way ANOVA followed by Bonferroni multiple comparison against vehicle control (*$p < 0.05$). **b**) Corresponding signal-to-noise (S/N) ratios of Z27743747 and Z3303614736 in the two assays. S/N ratio was calculated as described in the methods section.